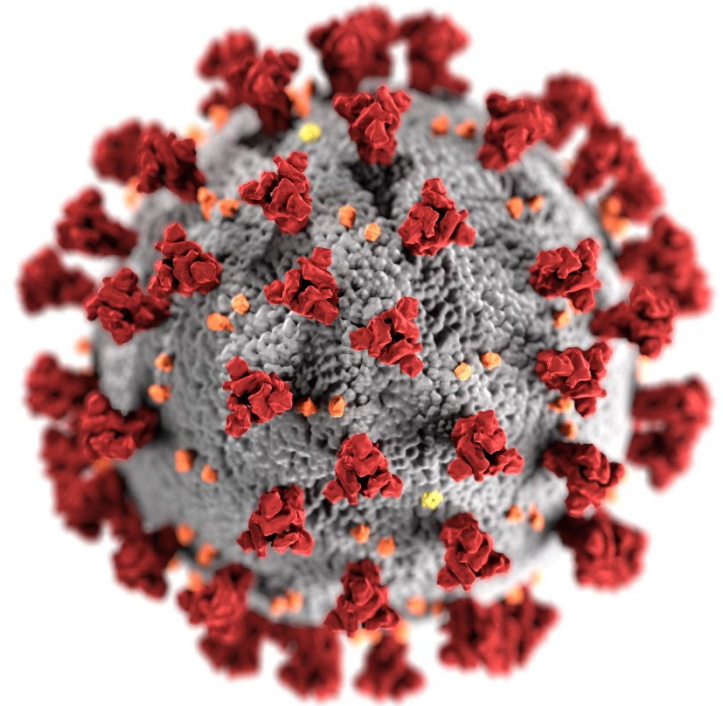


Wastewater-based variant tracking for SARS-CoV-2

COVID-19 Genomic Epidemiology Toolkit: Module 2.7

Brian Lowe
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City of Burlington, VT



cdc.gov/coronavirus

Toolkit map

Part 1: Introduction

- 1.1 What is genomic epidemiology?
- 1.2 The SARS-CoV-2 genome
- 1.3 How to read phylogenetic trees
- 1.4 Emerging variants of SARS-CoV-2

Part 2: Case Studies

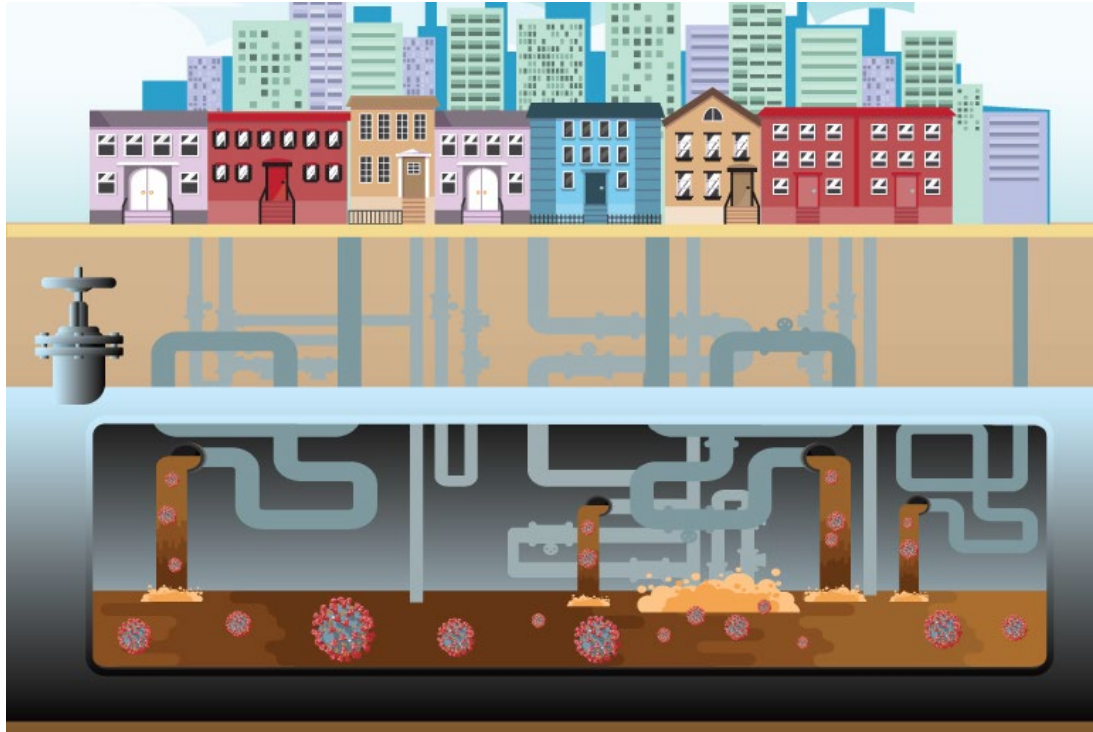
- 2.1 SARS-CoV-2 sequencing in Arizona
- 2.2 Healthcare cluster transmission
- 2.3 Community transmission
- 2.4 Superspreading event
- 2.5 Confirming reinfection
- 2.6 Detecting & prioritizing variants
- 2.7 Wastewater-based variant tracking**

Part 3: Implementation

- 3.1 Getting started with Nextstrain
- 3.2 Getting started with MicrobeTrace
- 3.3 Phylogenetics with UShER
- 3.4 Walking through NextStrain trees
- 3.5 Public genome repositories
- 3.6 Sequencing strategies



Wastewater surveillance - Strategic sampling and testing for pathogens or other health targets in wastewater to better understand disease burden within a community.



**Pooled
community
sample**

Advantages of SARS-CoV-2 wastewater surveillance

- Efficient, pooled sample representing hundreds or millions of people
 - Sampling within collection network for added granularity
- Independent of healthcare-seeking behavior or access
 - Captures symptomatic and asymptomatic infections
- Fast, community-level measure of infection burden
 - Results in 5-7 days vs 2 weeks for clinical case counts, hospitalizations

CDC National Wastewater Surveillance System



CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People™

Search Healthy Water

[Advanced Search](#)

Waterborne Disease & Outbreak Surveillance Reporting

Waterborne Disease & Outbreak Surveillance

Waterborne Disease & Outbreak Surveillance

National Wastewater Surveillance System (NWSS)

A new public health tool to understand COVID-19 spread in a community

The Centers for Disease Control and Prevention (CDC) and the US Department of Health and Human Services (HHS), in collaboration with [agencies throughout the federal government](#), are initiating the National Wastewater Surveillance System (NWSS) in response to the COVID-19 pandemic. The data generated by NWSS will help public health officials to better understand the extent of SARS-CoV-2 (the virus that causes COVID-19) infections in communities.

CDC is currently developing a portal for state, tribal, local, and territorial health departments to submit wastewater testing data into a national database for use in summarizing and interpreting data for public health action. Participation in a national database will ensure data comparability across jurisdictions.

Data from wastewater testing are not meant to replace existing COVID-19 surveillance systems, but are meant to complement them by providing:

On This Page

- What is wastewater surveillance for COVID-19?
- What are the advantages of wastewater infectious disease surveillance?
- Is wastewater surveillance right for my community?
- How can I implement wastewater-based disease surveillance?
- How do I become engaged in NWSS?

Waterborne Disease & Outbreak Surveillance

- Waterborne Disease & Outbreak Surveillance
- Detecting and Investigating Waterborne Diseases and Outbreaks
- Systems for Tracking Waterborne Disease and Outbreaks
- Wastewater Surveillance**
- Sampling Strategy: Where, How, and What to Sample
- Federal Coordination and Partnering for Wastewater Surveillance
- Testing Methods
- Data Reporting and Analytics

Website resources:

- Program overview
- Sampling strategies
- Testing methods
- Data reporting, analytics
- Data interpretation for public health action
- Targeted wastewater surveillance
- Low-resource wastewater system
- Limitations

www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html

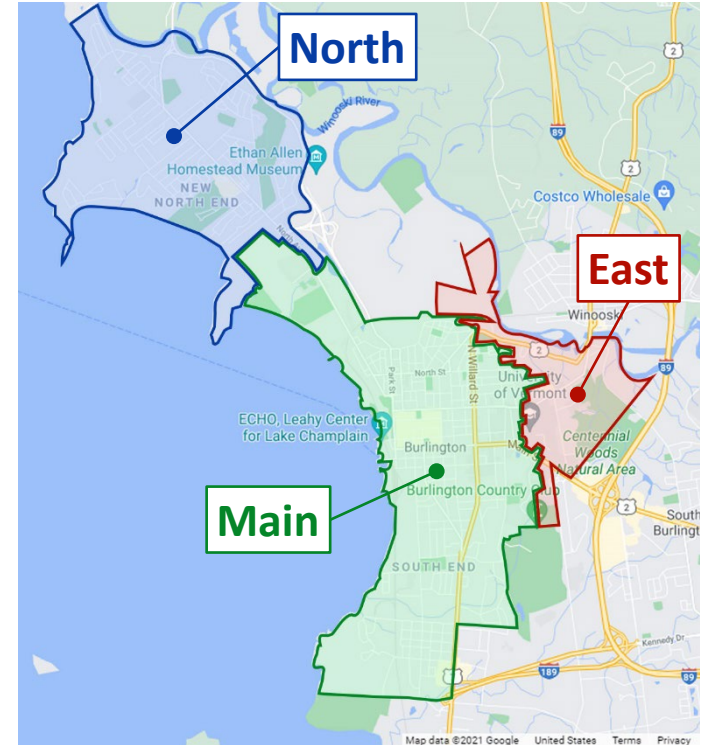
Burlington, VT wastewater surveillance

- In summer 2020, city officials had two main concerns:
 - Potential rise in caseloads as school resumed and college students return to campus from across the United States
 - University of Vermont students make up >20% of the local population
 - Lack of equitable access to limited testing resources
 - Potential lag in case identification, particularly among vulnerable groups

- Relative low-cost to implement, minimal staff time

Burlington, VT wastewater surveillance design

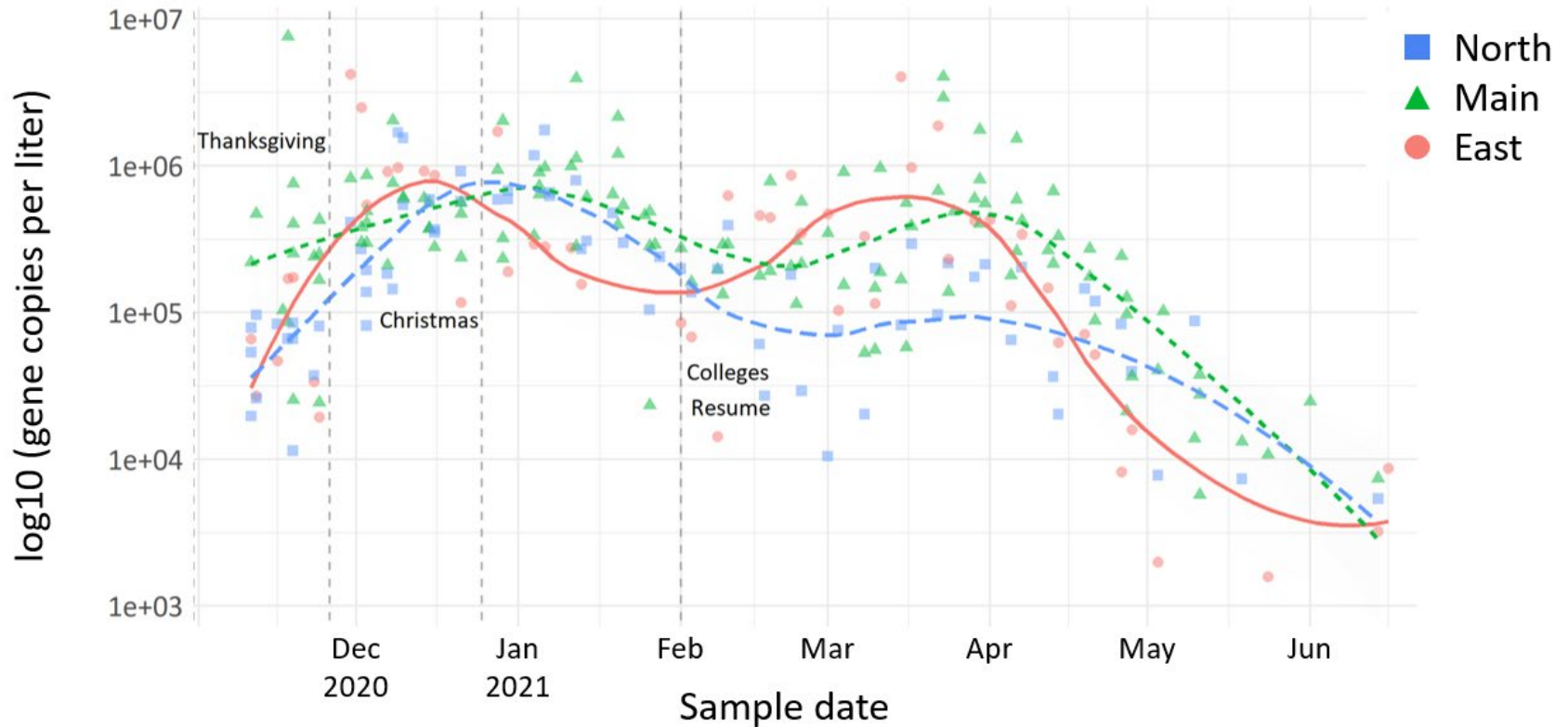
- Wastewater collection system separated into sewersheds of three treatment plants:
 - North, East, Main
 - Baseline sampling 2x per week
- Sub-sewershed sampling locations within Main (3), North (2) catchment areas
- All samples collected as 24hr composites due to normal flow variation
- Overnight shipment to commercial laboratory for droplet digital RT-PCR testing of N1 region
- Turnaround time: 2 days



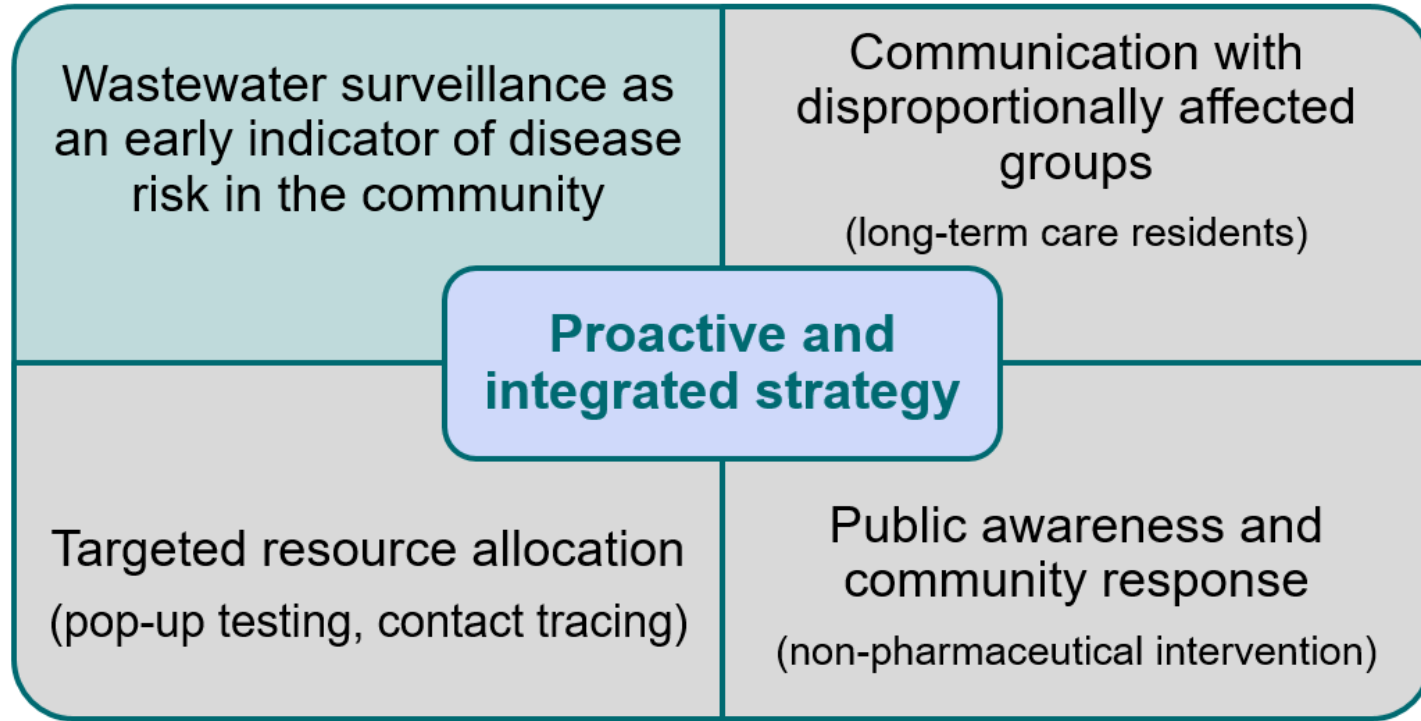
Burlington, VT wastewater surveillance evaluation

- Scenarios in consultation with Vermont Department of Health (VT DOH):
 1. Initial non-detection
 2. Sustained non-detection + low clinical case reporting, similar rate as August 2020
 3. Sustained non-detection + high clinical case reporting, 5-10x higher (termination)
 4. Low detection at wastewater treatment plant(s)
 5. Initial high detection at wastewater treatment plant(s)
 6. Increasing concentration for 2+ weeks
- August 2020 – SARS-CoV-2 RNA detected sporadically
- November 2020 – SARS-CoV-2 RNA detection, clinical cases increased
 - Begin sub-sewershed sampling 1x per week

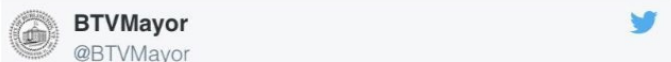
SARS-CoV-2 RNA detection in Burlington wastewater



Public health responses to increasing wastewater detection trends



Wastewater surveillance informed action

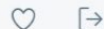
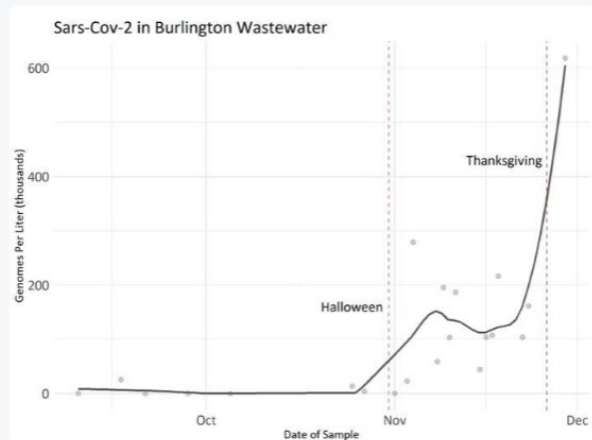


BTVMayor

@BTVMayor

The City's latest Covid-19 wastewater readings are back, and they show a major increase across the city. "I hope all Burlingtonians will look at this graph and see what I see: a call to action." - Miro

burlingtonvt.gov/Press/mayor-mi...



Dec 3, 2020

COVID-19 POPUP TESTING LOCATIONS

Week of 11/15-11/21

Availability of dates or testing subject to change

Pre-Registration Only

11/18 5PM-8PM NEW!

Robert Miller Center (Indoors)
130 Gosse Ct.
Burlington, VT 05408

11/21 9AM-12PM NEW!

O.N.E. Community Center (Outdoors)
108 Cherry St.
Burlington, VT 05401

Walk-Ins

11/19 9AM-3PM

Robert Miller Center
130 Gosse Ct.
Burlington, VT 05408

To schedule an appointment:

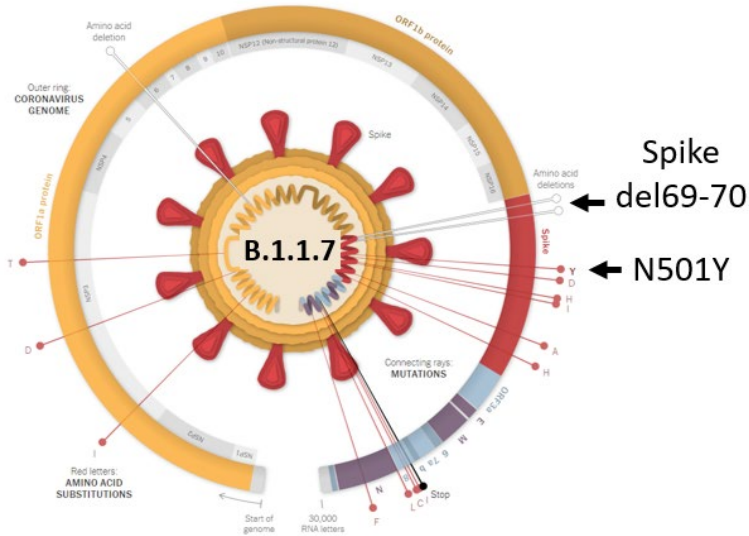
<https://phreesia.me/BurlingtonPopUp>

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Interpreters Available



Expanded surveillance – variant mutation tracking



- **December 2020:** First reports of Variant of Concern (VOC) B.1.1.7 (Alpha)
 - Potential increased transmissibility
- **Added RT-PCR targets for two spike mutations:** del69-70, N501Y
 - N501Y also found in other VOCs: B.1.351 (Beta), P.1 (Gamma)
- **January 13, 2021:** Testing 1x per week

More Toolkit modules on variants:

- Module 1.4 – Emerging variants of SARS-CoV-2
- Module 2.6 – Detecting and prioritizing variants

Image from *The New York Times* “Inside the B.1.1.7 Coronavirus Variant”

<https://www.nytimes.com/interactive/2021/health/coronavirus-mutations-B117-variant.html>

Amino acid abbreviations: https://www.ncbi.nlm.nih.gov/Class/MOLCOURSE/Modules/MolBioReview/iupac_aa_abbreviations.html

SARS-CoV-2 variant tracking in wastewater

- Wastewater may be useful for variant detection and tracking
 - Targeting mutations by RT-PCR (Burlington, VT)
 - Direct clinical specimen sequencing
- Public health interpretation challenges:
 - Mutation detection by RT-PCR does not indicate presence of a particular variant
 - Target mutations must be known, specific and novel mutations cannot be identified
 - Fragmented SARS-CoV-2 genomes from multiple, circulating variants present in community become mixed in wastewater
- Quantitatively monitoring mutations associated with known variants can provide information on trends in variant infections within a community.

Variant detection and clinical sequence confirmation

Jan

13

Burlington wastewater surveillance begins targeting two spike mutations associated with B.1.1.7

Feb

10

N501Y and del69-70 mutations detected by RT-PCR in Main sewershed sample collected on February 8

Feb

11

Public announcement and actions

- Burlington and Vermont released coordinated press statements to advise the public on the detection and evolving risk, Burlington held press conference
- Distributed high-quality masks to essential workers and delivered HEPA air purifiers to local businesses
- Alerted the local university where new cases were concentrated

Variant detection and clinical sequence confirmation

Feb 11 Vermont Department of Health (VT DOH) requested specimens from Burlington residents within impacted Main plant sewershed.

Feb 16 and Feb 23 Clinical specimens from Burlington residents sent to MASPHL for sequencing, no variants of concern identified.

Feb 21 East plant sample collected for testing

Feb 25 Variant mutations detected by RT-PCR in the East sewershed which serves most of the University of Vermont (UVM) campus

Variant detection and clinical sequence confirmation

Feb

26

VT DOH requests clinical specimens from UVM Student Health Clinic. Specimens from suspected symptomatic cases with a positive antigen test diverted to the VT DOH for diagnostic RT-PCR and evaluation for sequencing.

Mar

1

VT DOH Laboratory confirmed S gene target failure (SGTF) commonly associated with B.1.1.7

Mar

2

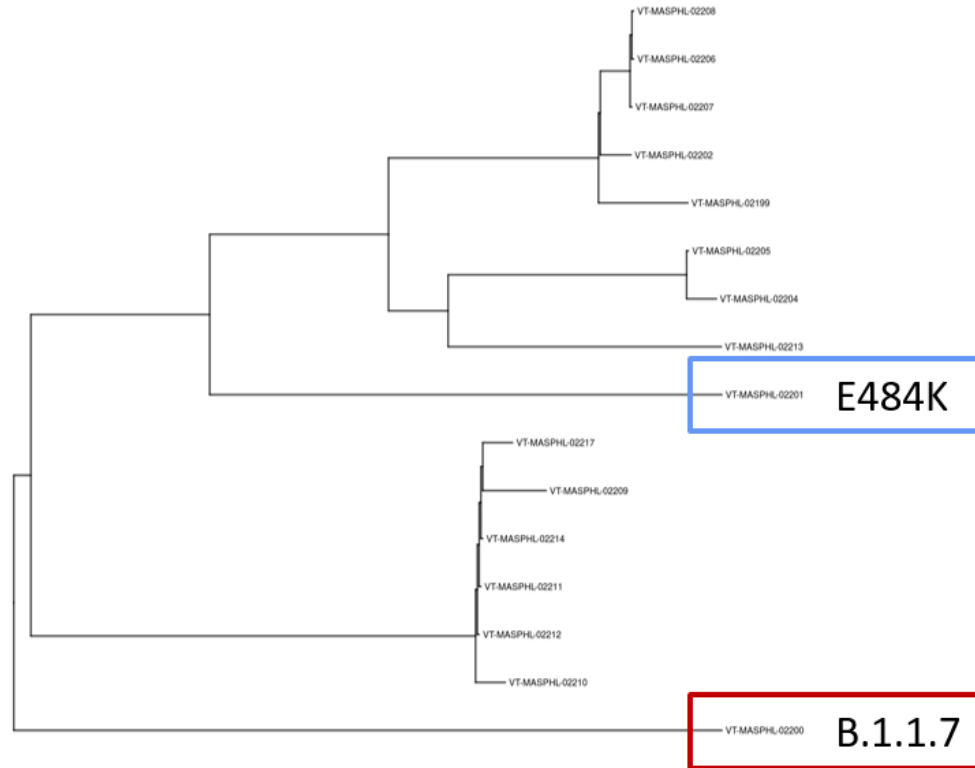
Specimen sent to MASP HL for sequencing

Mar

8

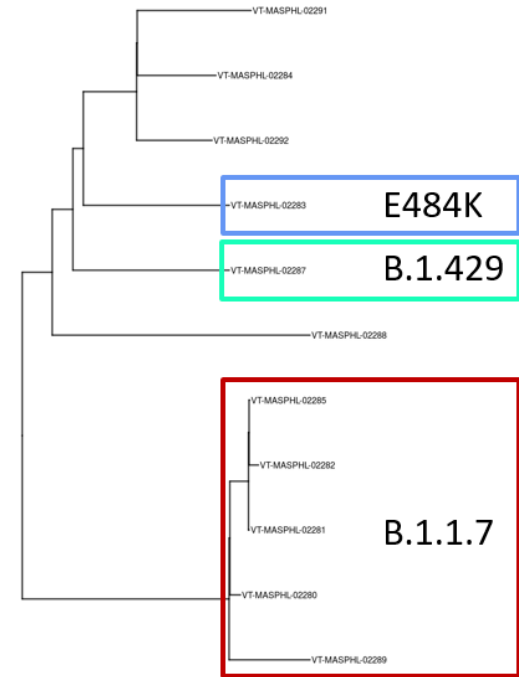
Confirmation of B.1.1.7 from clinical specimen

B.1.1.7 clinical sequence confirmation



B.1.1.7 clinical sequence confirmation

- B.1.1.7 identified in other Burlington clinical specimens in weeks that followed.
- VT DOH responded by:
 - Informing local guidance for control measures including at the University of Vermont
 - Conducting state-wide educational outreach about variants
 - Focusing on detecting variants in other areas experiencing high rates of disease – critical given limited sequencing capacity



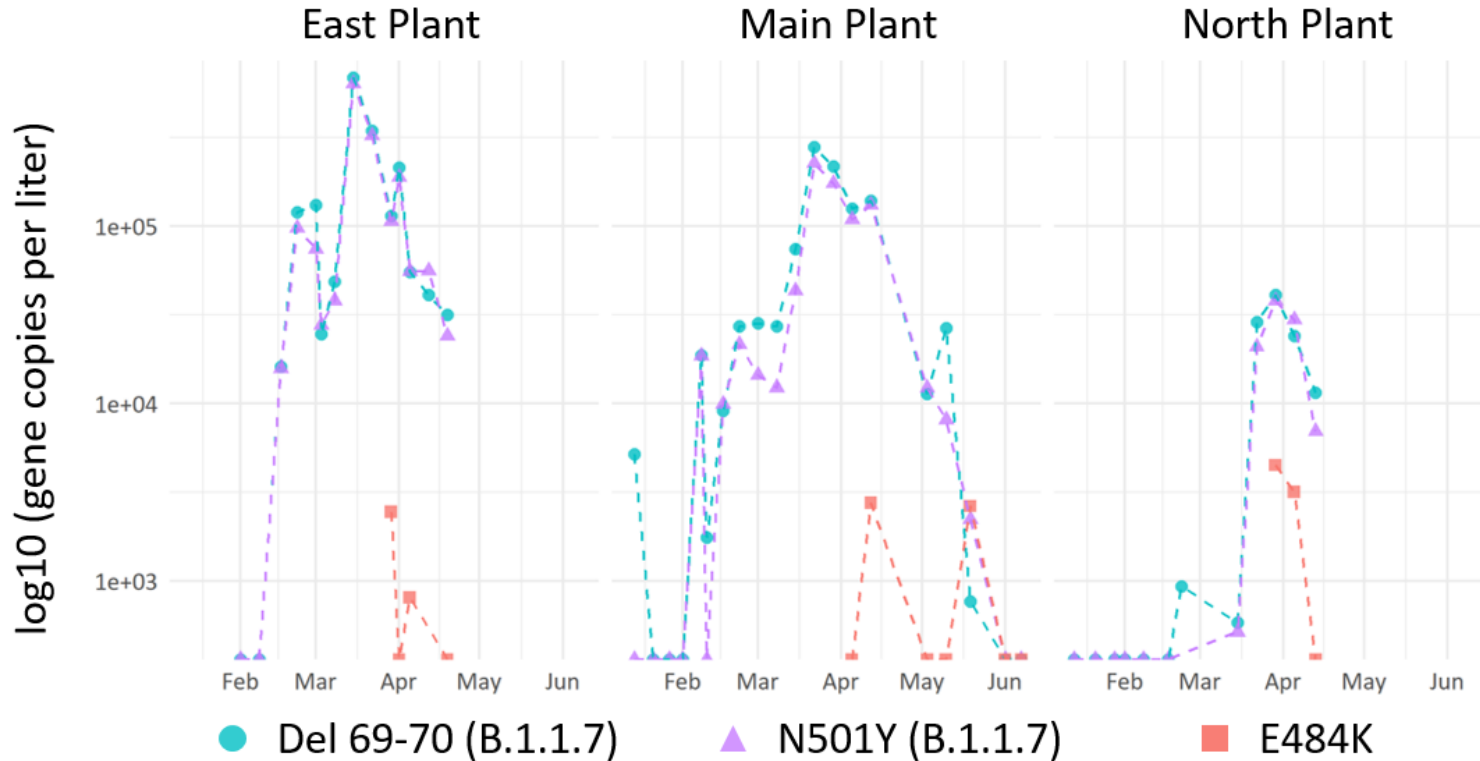
Tree by Andrew Lang, MASP HL

https://nextstrain.org/groups/spheres/ncov/vermont?c=pango_lineage&f_division=Vermont&p=full

Actions taken at UVM before B.1.1.7 confirmation

- UVM Student Health suspected B.1.1.7 on campus given the presence of variant mutations in wastewater and detection in neighboring states.
- Responded quickly by:
 - Increasing isolation and quarantine housing capacity
 - Separating isolation and quarantine housing into different buildings (rather than separate floors)
 - Increasing contact tracing capacity
 - Added staff
 - Incentivized evening and weekend coverage
 - Increasing student testing from once to twice per week

Monitoring frequency of variant spike mutations, 2021



Summary

- Wastewater surveillance provides early detection of increased SARS-CoV-2 infection within the community
 - Fast turnaround time facilitates response
 - Part of integrated approach to disease control
- Improved detection of B.1.1.7 (Alpha) by targeting defining mutations
 - Early warning of potential increased transmissibility
 - Detected mutations 4 weeks before variant confirmation by sequencing
 - Helped prioritize clinical specimens for sequencing, given limited capacity

Acknowledgements

- Multi-agency collaboration
 - City of Burlington
 - Dept of Innovation and Technology
 - Water Division, Dept of Public Works
 - Vermont Department of Health
 - Massachusetts State Public Health Lab
 - GoAigua, [GoAigua - IDRICA](#)
 - GT Molecular, www.gtmolecular.com
 - University of Vermont Student Health

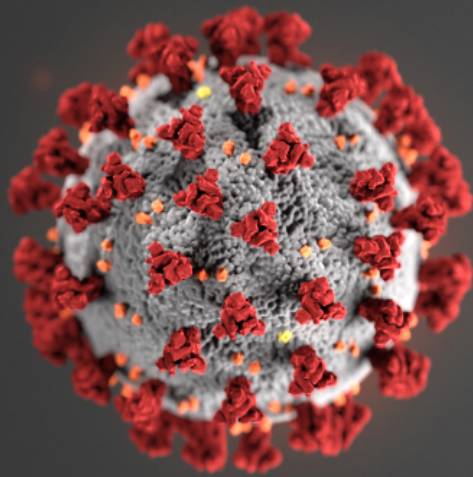


Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

Learn more

- Other modules
 - The SARS-CoV-2 genome – Module 1.2
 - Emerging variants of SARS-CoV-2 – Module 1.4
 - Detecting and prioritizing variants – Module 2.6
- COVID-19 Genomic Epidemiology Toolkit
 - Find further reading
 - Complete a feedback survey
 - Subscribe to receive updates on new modules as they are released
 - go.usa.gov/xAbMw





For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

